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Availability of iron and zinc in commercial infant cereals

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AVAILABILITY OF IRON AND ZINC IN COMMERCIAL INFANT CEREALS

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ABSTRACT

AVAILABILITY OF IRON AND ZINC IN

COMMERCIAL INFANT CEREALS

By Nasim Nazari

Three commercial infant cereals (rice, oatmeal, and mixed grain) were analyzed for phytate, iron, and zinc. Phytic acid was determined colorimetrically. Iron and zinc were analyzed using the AOAC atomic absorption spectrophotometeric method. The molar ratios of phytate: iron, phytate: zinc and iron: zinc in the samples were calculated and compared to the molar ratio values in the literature to evaluate the availability of iron and zinc in these products.

Phytic acid concentrations were 2.31 and 1.41 mg/g in the mixed grain, and rice cereals, respectively, and it was undetectable in the oatmeal cereal. The molar ratios of phytate: iron were 0.89 and 0.49 and the values for phytate: zinc were 3.18 and 1.75 in the mixed grain, and rice cereals, respectively. The molar ratios of iron: zinc were 3.55, 3.21, and 3.58 for the mixed grain, oatmeal, and rice cereals, respectively. Based on these values iron availability is not influenced by phytic acid and zinc levels in these products. In addition, zinc availability is not impaired by phytic acid level in any of the products; however, the iron levels might affect zinc availability in all the samples.

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PREFACE

The following thesis is written in two publication styles. Chapters 1 and 3 are written according to the guidelines outlined in the Publication of the American Psychological Association, 5th edition, 2001. The second chapter is written in journal format and will be submitted to Journal of Food Science.

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CHAPTER 1

INTRODUCTION AND REVIEW OF LITERATURE

Introduction

Iron storage of full-term, normal birth weight, breast-fed babies is sufficient for the first six month of their lives. After this time, they need to be supplied with readily absorbable iron sources for their cognitive performance and rapidly expanding blood volume. The World Health Organization (WHO) recommends introduction of complementary foods, in addition to breast milk, at the age of six months. Exclusive breastfeeding, after six months of age, may lead to iron deficiency anemia especially in low birth weight infants and zinc deficiency (Brown et al., 2002) which may lead to growth failure and delayed cognitive performance. Recommended Dietary Allowance (RDA) of iron for infants six months to one year is 11 mg/day and that of zinc is 3 mg/day (Brown et al., 2002).

Generally, cereal based foods are one of the first semi-solid foods to be introduced into infants' diets around the globe. In developed countries, they are commercially available in markets, although in developing countries, they are usually home-prepared. Cereal based foods are a good source of energy and are easily digestible; however, since they are primarily based on grains such as wheat and oats, they usually contain high levels of phytic acid, a natural antinutritional factor.

Phytic acid or inositol hexaphosphate is a natural component of many plants as the primary storage form of phosphate. It is found mostly in grains, legumes, nuts, oilseeds, tubers, and organic soils (Oatway, Vasanthan, & Helm, 2001). The unique structure of phytic acid enables it to form insoluble complexes with many compounds such as protein, starch, and bivalent metal ions such as iron (Glahn & Wortley, 2002), zinc (Bosscher et al., 2001), magnesium (Bohn, Davidsson, Walczyk, &Hurrell, 2004), and calcium (Oatway et al.,

2001) in foods; consequently, reducing or inhibiting their bioavailabilities. The extent of the inhibitory effect of phytic acid depends on several factors such as the ratio of phytate to these minerals, and environmental factors such as pH.

There are several ways to counteract the negative effect of phytic acid such as using phytase to break down phytic acid, using ascorbic acid to enhance iron absorption, and replacing high extraction flours with low extraction flours in which the concentration of phytic acid is lower.

Another factor that might affect mineral availability in infant cereals is the effect of co-fortification. Some infant cereals are fortified with iron and zinc. These two minerals compete for absorption; therefore, high intakes of one can reduce the absorption of the other. This effect has been shown to be greater when iron and zinc are provided in the form of supplements (Lind and others 2003). The percentage of inhibition could vary as a function of their chemical forms and their ratio in the food (Herman et al., 2002; Romana, Lonnerdal & Brown, 2003).

Objective

The objective of this study was to determine the molar ratios of phytic acid to iron and zinc and iron to zinc in commercial rice, oatmeal, and mixed grains infant cereals and to evaluate availability of iron and zinc in these products based on the molar ratio values reported in the literature.

Significance of the study

Commercial infant cereals usually made of grains such as rice, wheat, and oats, are one of the first semi-solid foods to be introduced to infants' diets. Moreover, infants need to be supplied by a rich source of iron after six month of age. Accordingly, many of the commercial infant cereals are fortified with iron and sometimes zinc. As a result, iron and zinc supplements are not recommended by pediatricians if infants are weaning to these fortified cereals. However, these minerals might not be absorbed at a satisfactory level due to several factors such as a high content of phytic acid in grains and the negative effect of iron and zinc on each others absorption. Since, molar ratio is a useful index of bioavailability, the molar ratios of phytic acid: iron, phytic acid: zinc and iron: zinc in three commercial infant cereals have been determined in this study to evaluate availability of iron and zinc in these products.

Review of Literature

Chemistry of phytic acid

Phytic acid or inositol hexaphosphate is a natural compound present in many plant foods especially legumes, nuts, oilseeds, and grains as a primary source of phosphorous (60-97%) (Oatway et al., 2001). Phytic acid concentration varies among plants and usually ranges between 1-2%. Phytic acid level can be affected by many factors such as genetics, environmental fluctuation, location, irrigation condition, type of soil, etc. (Reddy, Pierson, Sathe & Salunkhe, 1989). Phytic acid concentrations in some foods, legumes, and grains are shown in Table 1. Phytic acid exists in food mainly as either phytate which is a calcium salt of phytic acid, or phytin which is a calcium and magnesium salt of phytic acid (Oatway et al., 2001). Other forms of inositol phosphates like tri-, tetra-, and penta-phosphate can be formed during food processing, digestion, or in the presence of phytase. Phytic acid complete hydrolysis results in inositol and inorganic phosphates (Oatway et al., 2001). The structure of phytic acid is shown in Figure 1. The unique structure of phytic acid enables it to form insoluble complexes with many compounds.

Antinutritional aspects of phytic acid

At a neutral pH range, the phosphate groups of phytic acid are negatively charged, allowing it to interact with positively charged compounds such as protein, starch, and many bivalent metal ions like iron (Glahn & Wortley, 2002), zinc (Bosscher et al., 2001), magnesium (Bohn et al., 2004), and calcium (Oatway et al., 2001) in foods, thereby, reducing or inhibiting their bioavailability, functionality, and digestion. Zinc has been found to be the mineral most affected by phytic acid because it forms the most stable and insoluble complexes with phytate (Oatway et al., 2001). The effect of other inositol phosphates other than inositol hexaphosphate on uptake inhibition of nutrients is not well understood. Persson, Turk, Nyman, and Sandberg (1999) found similar mineral binding capacity for inositol hexaphosphate (IP6), inositol pentaphosphate (IP5), inositol tetraphosphate (IP4), and inositol triphosphate (IP3) when conditions were simulated to the pH range of the upper duodenum where mineral absorption takes place although the binding strength was lower for the lower inositol phosphates (IP4 and IP3), and the strength was different between different minerals. In another study by Sandberg et al. (1999), IP_5 was found to have an inhibitory effect on iron absorption, whereas IP_3 and IP_4 in isolated forms didn't have such an effect. However, in processed food, IP4 and IP3 contributed to a negative effect on iron absorption, presumably by the binding between

Table 1. Phytic Acid Content in Food, Legume, and Grain Products as Determined by HPLC

	Phytic Acid (%)	Relative (%)			
		IP_6	IP_5	IP_4	IP_3
Barley (Betzes)	0.56	69	22	6	3
Beans (Great Northern)	1.12	93	5	2	0
Beans (Lima)	0.84	87	9	4	0
Beans (Navy)	1.09	91	6	4	0
Beans (Pinto)	0.93	90	5	5	0
Buckwheat	1.08	91	8	0.6	0.4
Corn steeped	0.08	74	20	3	3
Corn (dent)	0.72	82	16	2	0
Corn cereal (toasted)	0.07	60	22	10	8
Corn flour	0.10	26	38	25	11
Millet	0.81	87	11	1	1
Oat cereal (toasted)	0.38	51	32	13	5
Oats	0.62	81	16	2	1
Rice bran (0.55% oil)	8.70	93	7	—	—
Rice bran (22% oil)	6.55	92	8	—	—
Rye	0.57	54	31	11	4
Sorghum	0.81	76	19	4	1
Soy concentrate	10.7	88	12	0	0
Soy flakes (defatted)	1.64	92	7	0.5	0.5
Spinach	0.008	100	0	0	0
Wheat (HRW)	0.77	88	11	1	0
Wheat (SWW)	0.65	83	15	2	0
Wheat cereal (toasted)	0.76	70	24	5	1
Wild rice	0.42	60	26	10	4

Note: From "HPLC separation and quantitation of phytic acid and some inositol phosphates in foods: problems and solutions" by J. Lehrfeld, 1994, *Journal of Agricultural and food chemistry*, 42, p.2729.

iron and the inositol phosphates. Therefore, the authors concluded that to improve iron absorption from cereals and legumes, degradation of inositol phosphates needed to achieve to less phosphorylated inositol phosphates than IP_3.

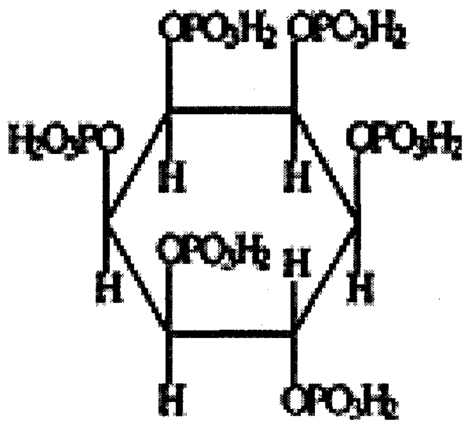

Figure 1: Basic Structure of Phytic Acid
Note: from "Phytic acid" by L. Oatway, T. Vasanthan, J.H. Helm, 2001, *Food Reviews International*, 17(4), p.420.

Phytic acid has been shown to inhibit iron absorption in infants and adults to a similar extent (Hurrell, Davidsson, Reddy, Kastenmayer & Cook, 1998). The type of cereal grain has little influence on iron bioavailability of infant cereals (Cook, Reddy, Burri, Juillerat & Hurrell, 1997); however, the inhibitory effect changes depending on the source of iron. The heme-iron has been shown to be significantly less affected than non-heme iron (Glahn & Wortley, 2002). One of the factors influencing the extent of the inhibitory effect of phytic acid on iron is its molar ratio to iron in food. Glahn and Wortley (2002) studied the effect of phytic acid on iron absorption in different molar ratios of $FeCl_3$ to phytic acid using an in vitro model. They found that at an equimolar ratio of phytic acid to iron, iron absorption decreased by 70%. Previous studies have recommended to decrease the negative effect of phytic acid on iron absorption, the molar ratio of phytate: iron in an iron-fortified food needs to decrease to <1, preferably <0.5 (Hurrell, 2003; Hurrell, Juillerat, Reddy, Lynch, Dassenko, & Cook, 1992).

Phytic acid also makes a very stable complex with zinc; consequently, reducing its bioavailability. The molar ratio of phytate to zinc is a good index to determine the inhibitory effect of phytates (Morris and Ellis, 1989). Previous research studies have conflicting results and there is no definitive conclusion regarding the maximum phytate/zinc molar ratio beyond which zinc status is significantly impacted negatively. This could be due to the influence of other factors like dietary calcium level. In vitro and animal experiment studies showed that high levels of dietary calcium augment the inhibitory effect of phytate on zinc because calcium reacts with phytate, and zinc binds to the precipitate (Morris and Ellis, 1980; Wise, 1995). In a research study by Lo, Settle, Steinke, and Hopkins (1981), the molar ratio of phytic acid: zinc 10:1 in soy protein isolates has been shown to improve zinc status of rats. Bosscher et al. (2002) studied the effect of phytic acid on zinc absorption in different ratios of phytic acid: zinc in infant foods using an in vitro model simulating the digestive conditions of infants, both younger and older than four months of age. They concluded that Zn availability was negatively affected when phytate: zinc ratio increased over 1.5. The negative effect of phytate was proven by finding the same bioavailability of Zn from a cow's milk based formula and soybean based formula when phytic acid was added to the cow's milk formula. On the other hand, a World Health Organization publication (1996) categorized diet with phytate: zinc molar ratios of < 5 as high in zinc bioavailability (50–55% absorption).

Methods of reducing the negative effect of phytic acid

Several methods of reducing the negative effect of phytic acid have been used in food industry. These include reduction of phytic acid by phytase or addition of some enhancers for mineral absorption (Hurrell, 2002b).

Phytic acid is commonly broken into myoinositol and other lower inositol phosphates in the infant cereal industry by either adding exogenous phytases that are produced by microorganisms or activating endogenous phytases in plants. The activities of these enzymes highly depend on pH with the highest strength at an acidic pH around 5.1(Oatway et al, 2001). Endogenous enzymes can be activated by soaking, germination, and fermentation (Hurrell, 2002a). Soaking legumes for several hours before processing activates phytases that are normally inactive in dry cereals due to low moisture content. Heat treatment like cooking, and replacing high extraction flours with low extraction flours which contain a lower concentration of phytic acid are the other ways to reduce or remove the negative effect of phytic acid (Oatway et al., 2001). Degradation of phytic acid has been shown to improve bioavailability of minerals (Zhao, Hao, Yin, Kastenmayor, & Barclay, 2003). However, phytate degradation has been shown to improve iron absorption from cereal porridges prepared with water but not with milk in adults (Hurrell, Reddy, Juillerat, & Cook, 2003).

Bioavailability of mineral in food can be increased by adding some enhancers. Ascorbic acid is one of the most popular enhancers of iron used in the food industry. Its mechanism is to reduce ferric iron to the ferrous state; thus, preserving its solubility at the pH of the duodenum (Conrad and Schade, 1968). It can boost non-heme iron absorption extensively at the ratio of 4.2:1 ascorbic acid: iron according to Davidsson et al. (1994). Evaluation of iron absorption from an iron fortified chocolate flavored milk drink by Davidsson et al. concluded that without adding ascorbic acid, the milk drink was a poor source of iron for children. Davidson et al. (1997) found similar results when cereals were made of low extraction flour and were fortified with ascorbic acid at the ratio of 2:1. The effect of ascorbic acid on iron absorption is so significant that even extensive reduction of phytic acid might not be necessary. Lind et al. (2003) evaluated the effect of extensive reduction in the phytate content of an infant cereal fortified with ascorbic acid on iron and zinc status in Swedish infants. Extensive reduction was carried out by scalding, soaking of samples at pH 4.5, along with applying endogenous phytases. Their results indicated very little long-term effect on the iron and zinc status of Swedish infants suggesting that a high concentration of ascorbic acid was one of the reasons for the enhanced iron absorption regardless of phytic acid content. Unfortunately, ascorbic acid doesn't increase the absorption of all forms of iron uniformly and it is very sensitive to heat during processing. According to Hurrell (2002), ascorbic acid affects iron absorption in all forms of ferrous sulfate, ferric ammonium citrate, ferrous fumarate, ferric orthophosphate and electrolytic iron. However, in order to be effective for electrolytic iron powders and the iron phosphate compounds, the amount of ascorbic acid should be increased.

Effect of co-fortification with iron and zinc

Simultaneous fortification of food with iron and zinc might not provide the satisfactory levels of iron and zinc to the body since they highly compete for absorption. This effect has been shown to occur to a greater extent when iron and zinc are provided simultaneously in the form of supplements (Sandstrom, Davidsson, Cederblad, & Lonnerdal, 1985; Whittaker, 1998). A similar effect has been observed in infants given aqueous solutions of single and combination supplements of those minerals (Lind et al., 2003). Few studies; however, have shown that iron fortified weaning food do not influence zinc absorption to the same extent in infants (Fairweather-Tait, Wharf & Fox, 1995) and in adults when iron and zinc are incorporated in a meal as fortifications (Davidsson, Almgren, Sandstrom, Hurrell, 1995). Molar ratio of iron to zinc in food is a good index for determining which one of these minerals is impairing the uptake of the other as well as the percentage of inhibition.

Glahn and Wortley (2002) studied the effect of zinc on iron uptake using an in vitro model. They found that addition of the $ZnCl_2$ at molar ratios of 1:0.5 and 1:1 iron to zinc decreased the iron absorption by 58 and 82%, respectively although at ratios less than 1:0.5 iron: zinc, iron absorption wasn't affected significantly. Zinc has been shown to impair iron absorption when its molarity is higher than that of iron in food (Rossander-Hulten, Brune, Sandstrom, Lonnerdal & Hallberg, 1991).

Excessive iron levels also affect zinc absorption negatively. However, heme-iron has been shown not to have such an effect on zinc absorption when ingested in a 3:1 Fe:Zn ratio with inorganic zinc according to Solomon and Jacob (1981). No significant iron inhibition on zinc absorption has been found at Fe: Zn ratio below 2:1 in rats although a dose dependent inhibition of zinc absorption has been indicated between molar ratios of 2:1 and 5:1 of Fe: Zn (Peres, Bureau, Neuville & Arhan, 2001). Similar results have been obtained by Solomons and Jacob (1981). They studied the effect of non-heme iron on zinc absorption in human intestine by measuring the concentration of plasma zinc after oral administration of these minerals in various ratios. Their results showed that at a ratio of Fe/Zn of 1:1, zinc absorption was inhibited slightly while Fe/Zn ratio of 2:1 and 3:1 substantially inhibited zinc uptake. Simultaneous supplementation of infant cereals with iron and zinc is not recommended by some researchers since it may not provide the satisfactory level of either zinc or iron for this age group of children (Lind et al., 2004).

Effect of chemical form of minerals

Recent studies have been shown that not only various chemical forms of minerals have diverse bioavailability but also different chemicals of the same mineral may have different inhibitory effects on the absorption of other minerals. There are currently five zinc salts listed as GRAS including zinc sulfate, zinc chloride, zinc gluconate, zinc oxide, and zinc stearate and 12 iron salts including elemental iron, ferrous ascorbate, ferrous fumarate, and ferric sulfate (Code of Federal Regulations).

Bioavailability of zinc sulfate and zinc oxide has been studied by Romana, Lonnerdal, and Brown (2003) indicating no difference in zinc status of adults when they were fed wheat products fortified with iron and either zinc sulfate or zinc oxide. In contrast, it has been found that the chemical type of zinc affects the iron absorption of foods co-fortified with these two minerals. Herman et al. (2002) studied the difference between using zinc sulfate and zinc oxide along with iron on iron status of Indonesian young children ages 4 to 8 when fortified in wheat flour dumplings. Zinc sulfate was found to have a greater inhibitory effect on iron absorption than zinc oxide although no significant difference was seen between the two zinc compounds on zinc status of the children.

Elemental iron which is one of the iron salts listed as GRAS exists in different forms such as carbonyl iron, reduced iron and electrolytic iron. Electrolytic iron is the common form of iron used in the cereal industry. Elemental iron has been shown to have lower bioavailability compared to other forms such as ferrous sulfate. Bioavailability of carbonyl iron versus ferrous sulfate was studied by Hallberg, Brune, and Rossander (1986). They found 5 to 20% less bioavailability for carbonyl iron than ferrous sulfate depending on the food served with wheat rolls. The results of a previous study have recently been confirmed by Walter, Pizarro, Abrams, Boy (2004). They incorporated elemental iron into white bread and fed it to children ages 5 to 7 years old. The results showed that bioavailability of iron elements was less than that of ferrous sulfate (<65%) depending on the particle size of elemental iron. Despite low bioavailability of elemental iron, it is the most popular chemical form of iron used for cereal fortification. Among the various types of elemental iron, electrolytic iron seems to be the best choice but double usage of it is recommended to provide a satisfactory level of iron absorption (Hurrell et al., 2002). There are many research studies underway to find a more stable iron form with higher bioavailability. Bothwell and MacPhail (2004) found that iron in the form of NaFeEDTA is less susceptible to environmental factors such as phytates and polyphenols because the iron moiety exchanges with the intrinsic food iron and the EDTA partially protects the iron in this common non-heme iron pool. NaFeEDTA also doesn't affect absorption of other minerals such as zinc.

CHAPTER 2

JOURNAL ARTICLE

Author's Title Page

Availability of Iron and Zinc in Commercial Infant Cereals

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ABSTRACT

Three commercially available infant cereal products (rice, oatmeal, and mixed grain) were analyzed for phytate, iron, and zinc. Phytic acid was determined colorimetrically. Iron and zinc were analyzed using the AOAC atomic absorption spectrophotometric method. Phytate: iron, phytate: zinc and iron: zinc molar ratios were calculated and compared to the molar ratio values in the literature to evaluate the availability of iron and zinc in these products.

Phytic acid concentrations were 2.31 and 1.41 mg/g in the mixed grain and rice cereals, respectively, and it was undetectable in the oatmeal cereal by this method. The molar ratios of phytic acid: iron were 0.89 and 0.49 in mixed grain, and rice cereals, respectively, and for the same cereals the ratios of phytic acid: zinc were 3.18 and 1.75, respectively. The molar ratios of iron: zinc were 3.55, 3.21, and 3.58 for mixed grains, oatmeal, and rice cereals, respectively. Based on these values iron availability is not influenced by phytic acid and zinc levels in these infant cereal products because the molar ratios of phytic acid: iron and iron: zinc were below the level of concern (<1 and >1, respectively). In addition, zinc availability would not be impaired by phytic acid level in any of the samples (molar ratio phytate: zinc <5); however, the iron levels might affect zinc availability in all the samples (molar ratio iron: zinc >2).

Keywords: bioavailability, infant cereals, phytic acid, electrolytic iron, zinc sulfate

Introduction

Iron storage of full-term, normal birth weight, breast-fed babies is sufficient for the first six months of their lives. After this time, infants need to be supplied with readily absorbable iron sources for their rapidly expanding blood volume and their cognitive performance. Generally, cereal based foods are one of the first semi-solid foods to be introduced into the infant's diet around the globe. Cereal based foods are a good source of easily digestible energy. However, since they are primarily based on grains such as wheat and oats, they usually contain high levels of phytic acid, a natural antinutritional factor. Phytic acid or inositol hexaphosphate is a natural component of many plants as the primary storage form of phosphate, which is mostly found in grains, legumes, nuts, oilseeds, tubers and organic soils. The unique structure of phytic acid enables it to form insoluble complexes with many bivalent metal ions like iron (Glahn and Wortley 2002), zinc (Bosscher and others 2001), magnesium (Bohn and others 2004), and calcium (Oatway and others 2001) in foods; consequently, reducing or inhibiting their bioavailabilities. The type of cereal grain has been shown to have little influence on iron bioavailability of infant cereals (Cook and others 1997). The extent of inhibitory action of phytate depends on several factors such as the ratio of phytate to those minerals, environmental factors like pH, the presence of other metal ions, and the source of minerals. For example, the inhibitory effect of phytic acid is much higher on non-heme iron than on heme-iron (Glahn and Wortley 2002). Phytic acid has been shown to inhibit iron absorption in infants and adults to a similar extent (Hurrell and others 1998).

Commercially available infant cereals are generally fortified with high levels of iron and zinc, which may negatively impact their uptake. Since zinc and iron compete for absorption, high intake of one can impair the absorption of the other. This effect has been shown to be higher when they are provided in the form of supplements (Lind and others 2003). The percentage of inhibition could vary as a function of the chemical form and their ratio in the food (Herman and others 2002; Romana and others 2003).

Various methods of reducing the negative effect of phytic acid have been studied. In the cereal industry, phytic acid is often reduced or removed by either adding exogenous phytases or activating endogenous phytases, or low extraction flours in which the phytic acid level has been decreased substantially are used (Hurrell and others 2003). Phytate degradation has been shown to improve iron absorption from cereal porridges prepared with water but not with milk in adults (Hurrell and others 2003). The addition of enhancers for mineral absorption such as ascorbic acid and EDTA is also very common to reduce the negative effect of phytic acid (Hurrell 2002b).

The objective of this study was to determine the molar ratios of phytic acid to iron and zinc and iron to zinc in commercial rice, oatmeal, and mixed grains infant cereals to evaluate availability of iron and zinc in these products based on the molar ratio values reported in the literature.

Materials and Methods

Materials

Analyses were performed on commercially available rice, oatmeal and mixed grains infant cereals, all fortified with electrolytic iron and zinc sulfate. Three boxes of each type of cereals were randomly chosen from a local supermarket. The mixed grain cereal consisted of wheat, rice, and oat flours. Nutrition information provided for all of the samples claimed to provide 45% and 20% of RDA for iron and zinc for infants up to one year in one 15 g serving size, respectively. The ascorbic acid levels in all the samples were reported to be zero. The chemical reagents used were certified A.C.S and were purchased from Fisher Scientific. Phytic acid used for making standard solutions was in the sodium salt form and was purchased from Sigma Chemical Company.

Sample preparation

The contents of the boxes were mixed by shaking and the specific weights of samples required for each determination were taken directly from the boxes. No further grinding was performed on the samples.

Phytic acid determination

Phytic acid was analyzed according to the method of Davies and Reid (1978) which was a modified version of the Holt method (1955). The principle of the method is based on the observation that ferric ions complex with thiocyanate ion to give a characteristic pink complex. In the presence of phytate, at pH 1-2, the ferric ions complex with phytate and do not react with thiocyanate. This results in the reduction of intensity of the pink color, which can be measured using a UV-spectrophotometer. Under these conditions an inverse relationship has been found over a range of phytate concentrations from 40 to 200 nmol (equals to 1.3 to 6.5 mg of phytic acid per gram of sample).

Procedure. Three 0.5 g samples of each infant cereal were extracted with 20 ml, 0.5 M-HNO_3 for 3 to 4 hr with continuous shaking at room temperature and then filtered through #1 Whatman filter paper. A 0.8 ml of aliquot extract was diluted with distilled water to a final volume of 1.4 ml in centrifugal tubes followed by addition of 1 ml of a solution of ferric ammonium sulfate containing 50 μg Fe, mixed and heated in a boiling water bath for 20 min. After cooling at room temperature, 5 ml of amyl alcohol was added followed by 0.1 ml of ammonium thiocyanate solution (100g/l), mixed and the mixture centrifuged at 3000 rpm for five minutes. Amyl alcohol layer was separated and absorbance was determined using Shimadzu (UV 160U) UV-Visible Recording Spectrophotometer at 465 nm against an amyl alcohol blank exactly 15 min after adding ammonium thiocyanate.

To determine the concentration of phytic acid in our samples, a standard curve was plotted based on the obtained absorbance of several known concentrations of standard solutions of phytate (40-200 nmol) which were prepared from 0.2 mM standard Na phytate stock solution in 0.5 M HNO_3. Then, the rest of the procedure was performed as described previously. The phytic acid content in the samples was calculated considering that the extract prepared from 0.5 g of the samples dissolving in 20 ml of 0.5 M-HNO_3, and the procedure was performed on 0.8 ml of the extracts. The phytic acid content of the oatmeal cereal was not detectable in the first extractions. Second extractions were prepared weighing 0.2 and 1 g of the sample to detect phytate if it is in higher or lower amounts detectable by this method. Both of the extractions showed intense pink color again after adding ammonium thiocyanate indicating that phytic acid was present in lower concentration than the level of the sensitivity of this method which was 1.3 mg/g of the sample.

Iron and zinc determination

Analyses of Zn and Fe were performed based on the AOAC official method 985.35, revision March 1998. The principle was destroying organic matrix by dry ashing in furnace, dissolving the obtained ashes in diluted acid, and determining the analyte by atomic absorption spectrophotometer using wavelength and flame specified for iron and zinc.

Procedure. Three 1.5 g of each type of the cereals were weighed to the nearest 0.1 mg into the previously acid washed crucibles, dried in 100° C oven overnight and ashed in muffle furnace at 525° C for 6 h. To ensure complete ashing, the residue obtained was cooled, and treated with 1 ml HNO_3 and then ashed for 2 more hours until a pinkish-white ash was obtained. The ash was dissolved in 5 ml 1N HNO_3 and quantitatively transferred into a 50 ml previously acid washed volumetric flask and diluted to volume with 1N HNO_3. The resulting solutions were analyzed for iron and zinc using Perkin-Elmer 2380 Atomic Absorption spectrophotometer at 248 and 214 nm, respectively.

Standard curves were constructed using standard solutions of iron (1.0-5.0 ppm) and zinc (0.25-1.0 ppm) prepared from commercially available certified AA standard solutions. Proper dilutions of the ash extracts were made to make the absorbance readings fall within the standard curves. From the standard curves, dilution factors and weight of the samples used, the levels of iron and zinc were then calculated.

Molar ratios

Molar ratios of phytic acid: iron, phytic acid: zinc and iron: zinc were calculated by first converting the levels of phytic acid, iron and zinc in mg/g to millimoles/g using molecular mass unit of 660.8 for phytic acid and atomic mass units of 55.85, and 65.38 for iron and zinc, respectively. Corresponding molar ratios were then obtained using these values.

Results and Discussion

Phytic acid levels in these commercially infant cereal products are shown in Table 2. Mixed infant cereals contains the highest phytic acid (2.31± 0.23mg/g) followed by rice infant cereal product (1.41± 0.09 mg/g). Phytic acid in the oatmeal infant cereals was not detectable by the method employed in this study. Since the lowest phytic acid 0standard in the analysis was 1.3 mg/g, it appears that the level of phytic acid in oatmeal I nfant cereal is considerably lower than 1.3 mg/g. Based on the reported phytic acid values for grains and cereals, phytic acid levels found in the three commercially infant cereal products are considerably low. This indicates that phytic acid level in these cereal products have been removed considerably before being used as ingredients. However, there is no available information as to the phytic acid reduction method used.

Iron and zinc contents of the three infant cereal products are also shown in Table 2. Iron levels in all the products were relatively the same and ranged from 0.22± 0.023, 0.24± 0.006, and 0.25± 0.006 mg/g in mixed grain, rice and oatmeal infant cereals, respectively. Similar trend in zinc levels were observed with oatmeal having highest level (0.093±0.002 mg/g) followed by rice infant cereal (0.081±0.001mg/g) and mixed grain infant cereal (0.071± 0.001).

Based on the RDA values for 6 to 12 months infants, 11 mg/day for iron and 3 mg/day for zinc (Brown and others 2002), about 0.33 mg of iron and 0.04 mg of zinc was expected in each gram of the samples. These levels were found to be higher for zinc and lower for iron, by about 200% and 70%, respectively, than the values indicated in the nutritional level of these products.

Iron availability

Phytic acid has been shown to have a negative effect on iron availability. The extent of its negative effect has been estimated by determining the molar ratio of phytic acid to iron present in food products. The molar ratios of phytic acid to iron were 0.89, and 0.49 in the mixed grains, and rice infant cereals, respectively (Table 5). The molar ratio of phytate: iron in oatmeal cereals was not determined because the phytic acid level in that sample was not detectable by the method employed in this study. Extensive in vitro and in vivo data in phytic acid: iron molar ratio and iron availability have been published. Based on these molar ratio values, it is apparent that the level of phytic acid in the three infant cereal products is relatively low to affect absorption of iron. The phytic acid: iron values for mixed grain and rice infant cereals were below 1.0 which is the level of concern according to Hurrell and others (1992) and Hurrell (2003). The previous studies recommend that to decrease the negative effect of phytic acid on iron absorption, the molar ratio of phytate: iron in an iron-fortified food be decreased to <1.0, preferably <0.5. Glahn and Wortley (2002) studied the effect of phytic acid on iron absorption in different molar ratios of FeCl3 to phytic acid using an *in vitro* model. They found decreased inhibition of iron by 70% at an equimolar ratio of phytic acid to iron.

Another factor that may influence the bioavailability of iron in a food product is the presence of other compounds such as zinc which compete for absorption with iron. The extent of this effect depends on their molar ratio in food (Crofton and others 1989). This effect has been shown to occur to a higher extent when both minerals are ingested simultaneously as supplements (Whittaker, 1998). The molar ratio of iron: zinc in the three cereal products was 3.21, 3.55, and 3.58 for rice, oatmeal, and mixed grain cereals, respectively (Table 5). Based on published data, zinc levels in the three infant cereal products have relatively negligible effect on iron absorption. Molar ratios of iron: zinc are below the level of concern in all the samples. Zinc has been shown to impair iron absorption when its molarity is higher than that of iron in food (Rossander-Hulten and others 1991). In vitro study by Glahn and Wortley (2002) on the inhibition of iron uptake by zinc showed a decline of 58% and 82% of iron absorption at molar ratios of 1:0.5 and 1:1 iron to zinc, respectively.

The other factor that can affect iron bioavailability is its chemical form. Studies have shown that the absorption rate of iron in food is influenced by the chemical form of iron present in the food. Infant cereals used in this study were fortified with the electrolytic form of iron. Despite the extensive usage of this form for fortifying cereals, it has been shown to have low bioavailability (Hallberg and others 1986) compared to other forms such as ferrous sulfate. Walter and others (2004) studied the absorbance rate of elemental iron by incorporating it into white bread fed to children ages 5 to 7 years old. Their results showed that the bioavailability of iron elements was less than that of ferrous sulfate. Overall, notwithstanding of low bioavailability of electrolytic iron, it still seems to be the best choice and the most popular chemical form used in cereal industry for fortification since it is inexpensive and has no impact on sensory attributes of food. However, to compensate for its low bioavailability, it is recommended to be applied at higher levels (Hurrell and others 2002). Therefore, in order for the infant cereal samples of this study to provide 45% daily value of RDA for iron (11mg) in each 15g serving size, more electrolytic iron would need to be added.

The form of zinc may also influence iron bioavailability when food is co-fortified with both of these minerals. The chemical form of zinc used in our samples was zinc sulfate, which may decrease iron absorption. Herman et al. (2002) studied the difference between using zinc sulfate and zinc oxide along with iron on iron absorption in Indonesian children ages 4 to 8 when fortified with wheat flour in dumplings. Zinc sulfate was found to have a greater inhibitory effect on iron absorption than zinc oxide.

Zinc availability

The presence of phytic acid has been shown to negatively affect zinc bioavailability, and the extent of this effect is measured by determining the molar ratio of phytic acid: zinc (Morris and Ellis 1989). The molar ratios of phytic acid to zinc in the samples used in this study were 3.18 and 1.75 for the mixed grains and rice infant cereals, respectively. Since phytic acid in the oatmeal cereal was not detectable the molar ratio of phytic acid: zinc was not determined. Phytate: zinc molar ratio values were below the level of concern (<5) in all of the samples according to WHO. Lo and others (1981) reported that the molar ratio of phytic acid: zinc at 10:1 in soy protein isolates improved zinc status in rats. Bosscher and others (2001) studied availability of Zn from infant foods containing different phytate contents using an *in vitro* model simulating the digestive conditions of infants. They found that Zn availability in small infants was negatively affected when the phytate: zinc ratio increased to over 1.5. This ratio value increased to approximately 8 for infants six months of age. Inhibition increased to 97.2% when the ratio of phytate to zinc was about 2.2. However, the World Health Organization (1996) categorizes a diet with phytate: zinc molar ratios of < 5 as high in zinc bioavailability (50–55% absorption).

An excessive iron level has been indicated to impair zinc absorption and their molar ratios ascertain the extent of inhibition (Peres and others 2001). Molar ratio of iron: zinc in the samples of this study as measured were 3.55, 3.21, and 3.58 in the mixed grain, oatmeal, and rice cereals, respectively. At these ratios, iron might impair zinc uptake since it is slightly higher than the level of concern reached in some of the previous researches. Solomons and Jacob (1981) studied the effect of non-heme iron on zinc absorption by measuring concentration of plasma zinc after oral administration of these minerals in various ratios. With zinc sulfate as the source of inorganic zinc, and ferrous sulfate as the source of non-heme iron, a ratio of Fe/Zn of 1:1 was found to slightly inhibit zinc absorption while Fe/Zn ratio of 2:1 and 3:1 substantially inhibited zinc uptake. These results are consistent with the results of a recent study by Peres and others (2001) which indicated that no significant iron inhibition on zinc absorption occurred at Fe:Zn ratio below 2:1 in rats although at higher ratios zinc uptake decreases significantly.

They found that at molar ratios between 2:1 and 5:1 of Fe: Zn, a dose dependent inhibition of zinc absorption occurred. On the other hand, Sandstrom and others (1985) found that iron did not impair zinc absorption at 2.5:1 ratio of iron: zinc. However, zinc absorption decreased significantly when ratio of iron: zinc increased to 25:1. It should be mentioned, however, that few studies have found that iron fortified weaning food do not influence zinc absorption to the same extent in infants (Fairweather-Tait and others 1995) and in adults when iron and zinc are incorporated in a meal as fortificant (Davidsson and others 1995).

Little information is available on the bioavailability of different types of zinc components. A recent study by Romana and others (2003) indicated no difference in zinc absorption between zinc sulfate and zinc oxide in adults when a wheat product was fortified with iron and either of the zinc salts. Similar findings were found by Herman and others (2002) when zinc sulfate and zinc oxide were incorporated into children's diets. Therefore, it is assumed that the chemical form of zinc in the samples of this research, zinc sulfate, would not impact its availability from food.

It is suggested that simultaneous fortification with iron and zinc in infant cereals may not provide the satisfactory level of zinc and iron for this group of children since these two minerals substantially compete for absorption. Similar finding has been indicated in a recent study when these two minerals were solved in water and given to infants as forms of single and combination supplements (Lind and others 2003). It has also been indicated that combined supplementation with iron and zinc at the iron: zinc molar ratio of 1.17:1 in a combined iron and zinc supplement had no significant effect on growth or development of the Indonesian infants from 6 to 12 months of age (Lind and others 2004).

Since the chemical form of iron used in the samples of this study has been shown to be approximately half as well absorbed compared to other types (Fomon 1987; Hallberg 1986; Walter and others 2004), doubling the use of it is recommended by some researchers (Hurrell and others 2002). However, we do not recommend that since it may further decrease zinc availability in the samples of this study. Instead, applying other types of iron compound like NaFeEDTA (Bothwell and MacPhail 2004) that has been shown to have high bioavailability, stability against inhibitors, and an inconspicuous effect on sensory properties of products is recommended. Meanwhile, bioavailability of iron and zinc can be substantially improved by incorporating ascorbic acid which is a strong enhancer of iron and zinc absorption (Conrad and Schade, 1968; Davidsson and others 1994).

Conclusion

Our finding shows that phytic acid levels in all the three infant cereals used in this study do not affect iron and zinc availability negatively. It is assumed that phytic acid in the samples of this study has been reduced extensively that its concentration is not affected iron and zinc absorption. However, further reduction of phytate in the rice and mixed grain cereals is recommended in order to assure zinc availability for infants younger than 6 months of age. The concentration of zinc in all of the samples was below the level beyond which iron availability is influenced. However, the iron concentration in all of the samples might influence zinc availability. No definite conclusion on bioavailability of iron and zinc can be reached due to an apparent discrepancy in the literature and the effect of the food compositions on the bioavailability of these minerals.

Finally, further in vivo research is recommended to evaluate the bioavailability of iron and zinc from the samples tested in this study.

Table 2. Phytic acid, Iron, and Zinc Concentrations in Mixed Grain, Oatmeal, and Rice Infant Cereals.

Samples[1]	Concentration of phytate (mg/g)	Concentration of Zn (mg/g)	Concentration of Fe (mg/g)
Mixed grain	2.31± 0.23	0.071± 0.001	0.22± 0.023
Oatmeal	Not detectable[2]	0.093±0.002	0.25± 0.006
Rice	1.41± 0.09	0.081±0.001	0.24± 0.006

[1]average of 3 samples ± SD

[2]not detectable by the Davis and Reid method at the concentration lower than 1.3 mg phytate per gram of the sample

Table 5. Molar Ratios of Phytic Acid to Iron and Zinc and Molar Ratio of Iron to Zinc in the Mixed Grain, Oatmeal, and Rice Infant Cereals.

Molar ratio	Mixed grain	Oatmeal	Rice cereal
Phytic acid: Zinc	3.18	NA*	1.75
Phytic acid: Iron	0.89	NA*	0.49
Iron: Zinc	3.55	3.21	3.58

* not applicable since phytic acid level in oatmeal cereal was not detectable by the Davis and Reid method

References

Association of Official Analytical Chemists. 1998. Official methods of Analysis of AOAC International (16th ed.) Gaithersburg, MD: Author.

Bohn T, Davidsson L, Walczyk T, Hurrell RF. 2004. Phytic acid added to white-wheat bread inhibits fractional apparent magnesium absorption in humans. Am J Clin Nut 79(3): 418-23.

Bosscher D, Lu Z, Jassens G, Van Caillie-Bertrand M, Robberecht H, De Rycke H, De Wilde R, Deelstra H. 2001. In vitro availability of zinc from infant foods with increasing phytic acid contents. Brit J Nut 86: 241-7.

Bothwell TH, MacPhail AP. 2004. The potential role of NaFeEDTA as an iron fortificant. Int J Vitam Nutr Res 74: 421-34.

Brown JE, Isaacs JS, Krinke UB, Murtaugh MA, Stang J, Wooldridge NH. 2002. Nutrition Through the Life Cycle. Belmont, CA: Wadsworth. 512 p.

Cook JD, Reddy MB, Burri J, Juillerat MA, Hurrell RF. 1997. The influence of different cereal grains on iron absorption from infant cereal foods. Am J Clin Nutr. 65(4):964-9

Crofton RW, Gvozdanovic D, Gvozdanovic S, Khin CC, Brunt PW, Mowat NA, Aggett PJ. 1989. Inorganic zinc and the intestinal absorption of ferrous iron [Abstract]. J Clin Nut 50: 141-4.

Conrad ME, Schade SG. 1968. Ascorbic acid chelates in iron absorption: a role for hydrochloric acid and bile [Abstract]. Gastr 55:35-45.

Davidsson L, Galan P, Kastenmayer P, Cherouvrier F, Juillerat MA, Hercberg S, Hurrell RF. 1994. Iron bioavailability studied in infants: the influence of phytic acid and ascorbic acid in infant formulas based on soy isolate. Pediatr Res 36: 816-22.

Davidsson L, Almgren A, Sandstrom B, Hurrell RF. 1995. Zinc absorption in adult humans: the effect of iron fortification. Br J Nutr 74(3):417-25.

Davidsson L, Walczyk T, Morris, A, Hurrell RF. 1998. Influence of ascorbic acid on iron absorption from an iron-fortified, chocolate-flavored milk drink in Jamaican children. Am J Clin Nut 67(5): 873-7.

Davidsson L, Ziegler E, Zeder C, Walczyk T, Hurrell R. 2005. Sodium iron EDTA [NaFe(III)EDTA] as a food fortificant: erythrocyte incorporation of iron and apparent absorption of zinc, copper, calcium, and magnesium from a complementary food based on wheat and soy in healthy infants. Am J Clin Nutr 81(1):104-9.

Davies NT, Reid H. 1979. An evaluation of the phytate, zinc, copper, iron and manganese contents of, and Zn availability form soya-based textured-vegetable-protein meat substitutes or meat-extenders. Brit J Nutr 41: 579-89.

Fairweather-Tait SJ, Wharf SG, Fox TE. 1995. Zinc absorption in infants fed iron-fortified weaning food. Am J Clin Nutr 62(4):785-89.

Fomon SJ. 1987. Bioavailability of supplemental iron in commercially prepared dry infant cereals. J Pediatr 110: 660-1.

Glahn RP, Wortley GM. 2002. Inhibition of iron uptake by phytic acid, tannic acid, and ZnCl2: Studies using an in vitro digestion/caco-2 Cell model. J Agric Food Chem 50: 390-5.

Hallberg L, Brune M, Rossander L. 1986. Low bioavailability of carbonyl iron in man: studies on iron fortification of wheat flour [Abstract]. Am J Clin Nut 43(1): 59-67.

Herman S, Griffin IJ, Suwarti S, Ernawati F, Permaesih D, Pambudi D, Abrams SA. 2002. Cofortification of iron-fortified flour with zinc sulfate, but not zinc oxide, decreases iron absorption in Indonesian children. Am J Clin Nut 76(4): 813-17.

Holt R. 1955. Studies on dried peas. I.- the determination of phytate phosphorus. J. Sci. Food Agric 6: 136-42.

Hurrell RF. 2002a. Enhanced iron absorption from cereal and legume grains by phytic acid degradation. ACS Symp Ser 816: 117-29.

Hurrell RF. 2002b. Fortification: Overcoming technical and practical barriers. J Nutr 132 (4suppl): 806s-12s.

Hurrell RF. 2003. Influence of vegetable protein sources on trace element and mineral bioavailability. J Nutr 133(9): 2973s-7s.

Hurrell R, Bothwell T, Cook,JD, Dary O, Davidsson L, Fairweather-Tait S, Hallberg L, Lynch S, Rosado J, Walter T, Whittaker P, SUSTAIN Task Force. 2002. The usefulness of elemental iron for cereal flour fortification: a SUSTAIN Task Force report. Sharing United States technology to aid in the improvement of nutrition. Nutr Rev 60: 391-406.

Hurrell RF, Davidsson L. Reddy M, Kastenmayer P, Cook JD. 1998. A comparison of iron absorption in adults and infants consuming identical infant formulas. Br. J. Nutr 79:31-6

Hurrell RF, Juillerat MA, Reddy,MB, Lynch SR, Dassenko, SA, Cook JD. 1992. Soy protein, phytate, and iron absorption in humans [Abstract]. Am J Clin Nut 56(3): 573-8.

Hurrell RF, Reddy MB, Juillerat MA, Cook JD. 2003. Degradation of phytic acid in cereal porridges improves iron absorption by human subjects. Am J Clin Nut 77(5):1213-9.

Lind T, Lonnerdal B, Persson L, Stenlund H, Tennefors C, Hernell O. 2003. Effects of weaning cereals with different phytate contents on hemoglobin, iron stores, and serum zinc: A randomized intervention in infants from 6 to 12 mo of age. Am J Clin Nut 78(1): 168-75.

Lo LS, Settle SL, Steinke FH, Hopkins DT. 1981. Effect of phytate: zinc molar ratio and isolated soybean protein on zinc bioavailability. J Nut 111: 2223-5.

Krebs NF, Westcott J. 2002. Zinc and breastfed infants: if and when is there a risk of deficiency? [Review]. Adv Exp Med Bio 503: 69-75.

Lind T, Lonnerdal B, Stenlund H, Ismail D, Seswandhana R, Ekstrom EC, Persson LA. 2003. A community-based randomized controlled trial of iron and zinc supplementation in Indonesian infants: interactions between iron and zinc. Am J Clin Nut 77(4): 883-90.

Lind T, Lonnerdal B, Stenlund H, Gamayanti IL, Ismail D, Seswandhana R, Persson A. 2004. A community-based randomized controlled trial of iron and zinc supplementation in Indonesian infants: effects on growth and development. Am J Clin Nut 80(3): 729-36.

Morris ER, Ellis R. 1989. Usefulness of the dietary phytic acid/zinc molar ratio as an index of zinc bioavailability to rats and humans [Abstract]. Biol Trace Elem Res. 19(1-2):107-17.

Morris ER, Ellis R. 1980. Effect of dietary phytate/zinc molar ratio on growth and bone zinc response of rats fed semipurified diets. J Nutr 110(5):1037-45.

Oatway L, Vasanthan T, Helm JH. 2001. Phytic acid. Food Rev Inter 17: 419-31.

Peres JM, Bureau F, Neuville D, Arhan P. 2001. Inhibition of zinc absorption by iron depends on their ratio [Abstract]. J Trace Elem Med Bio 15: 237-41.

Romana DL, Lonnerdal B, Brown KH. 2003. Absorption of zinc from wheat products fortified with iron and either zinc sulfate or zinc oxide. Am J Clin Nut 78(2): 279-83.

Rossander-Hulten L, Brune M, Sandstrom B, Lonnerdal B, Hallberg L. 1991. Competitive inhibition of iron absorption by manganese and zinc in humans Am J Clin Nut 54(2): 152-6.

Sandstrom B, Davidsson L, Cederblad A, Lonnerdal B. 1985. Oral iron, dietary ligands and zinc absorption. J Nutr 115(3): 411-14.

Solomons NW, Jacob RA. 1981. Studies on the bioavailability of zinc in humans: effects of heme and nonheme iron on the absorption of zinc [Abstract]. Am J Clin Nut 34(3): 475–82.

Walter T, Pizarro F, Abrams SA, Boy E. 2004. Bioavailability of elemental iron powder in white wheat bread [Abstract]. Eur J Clin Nutr 58: 555-58.

Wise A. 1995. Phytate and zinc bioavailability [Review]. Int J Food Sci Nutr 46(1):53-63.

Whittaker P. 1998. Iron and zinc interactions in humans. Am J Clin Nut 68(2): 442S-446S.

World Health Organization. Trace elements in human nutrition and health. Geneva: WHO, 1996.

CHAPTER 3

SUMMARY AND RECOMMENDATIONS

Summary

Infant cereals are usually made of grains in which a considerable amount of phytic acid is present. Phytic acid has long been identified as an inhibitor of many of the minerals such as iron and zinc. In addition, the simultaneous presence of iron and zinc beyond certain levels may influence their bioavailability. The objective of this study was to determine phytate, iron, and zinc concentrations in commercially available mixed grain, oatmeal, and rice infant cereals to determine availability of iron and zinc in them based on their molar ratios. The content of phytic acid was determined colorimetrically. Analyses of Zn and Fe were performed based on the AOAC official method 985.35. Phytate: iron, phytate: zinc and iron: zinc molar ratios were calculated and compared to the molar ratio values in the literature to evaluate the availability of iron and zinc in these products.

Phytic acid concentrations in the mixed grain and rice infant cereals were 2.31± 0.23 and 1.41± 0.09 mg/g, respectively. Phytic acid was undetectable in the oatmeal infant cereal by the Davies and Reid method. This method can only detect phytate at the range of 1.3 to 6.5 mg/g of the samples. Therefore, second extractions of the oatmeal cereal were prepared weighing 0.2 and 1 g of the sample to detect phytate if it is in higher or lower amounts detectable by this method. Both of the extractions showed intense pink color again after adding ammonium thiocyanate indicating that phytic acid was present in lower concentration than the level of the sensitivity of this method which was 1.3 mg/g of the sample.

Iron and zinc concentrations were 0.22± 0.023, 0.25± 0.006, 0.24± 0.006 mg/g and 0.071± 0.001, 0.093±0.002, 0.081±0.001 mg/g in the mixed grain, oatmeal, and rice infant cereals, respectively. These levels were found to be higher and lower for zinc and iron, by about 200% and 70%, respectively, than what was reported on the labels in all samples.

Molar ratios of phytic acid to iron in the samples were calculated 0.89, and 0.49 for the mixed grain and rice infant cereals, respectively. It was not possible to calculate this ratio in the oatmeal sample because phytic acid was not detectable in this sample by this method. On the basis of previous studies, the concentration of phytic acid in the samples analyzed would not influence the absorption of iron because the molar ratio of phytic acid: iron in all samples was below the level of concern (<1) (Hurrell et al., 1992; Hurrell, 2003). The molar ratio of iron: zinc in the samples of this study was found to be 3.21, 3.55, and 3.58 for rice, oatmeal, and mixed grain, respectively (Table 5). Considering the results of previous studies, zinc concentrations in the samples of this study may not affect iron uptake negatively since the molar ratio of iron: zinc was higher than the level that could affect iron absorption (>2).

The molar ratios of phytic acid to zinc in the samples analyzed were 3.18, 0, and 1.75 for the mixed grain, oatmeal, and rice infant cereals, respectively. The phytate: zinc ratio was below the level of concern in rice and oatmeal cereals (5) according to WHO; however, the results may not be conclusive due to significant variations in previous studies. As the presence of zinc may decrease iron absorption, an excessive iron level has also been shown to inhibit Zn absorption. The extent of this inhibition is in proportion to their molar ratios. The molar ratio of iron: zinc in the samples of this study as measured were 3.55, 3.21, and 3.58 in mixed grain, oatmeal, and rice cereals, respectively. At these ratios, iron might impair zinc uptake since it is slightly higher than the level of concern (2) (Rossander-Hulten et al., 1991; Glahn and Wortley, 2002). We concluded that simultaneous supplementation with iron and zinc in infant cereals may not provide the satisfactory level of zinc for this group of children. Our recommendation is consistent with that of Lind et al. (2003) study when these two minerals were solved in water and given to infants as forms of single and combination supplements. It has also been indicated that combined supplementation with iron and zinc at the iron:zinc molar ratio of 1.17:1 in a combined iron and zinc supplement had no significant effect on growth or development of the Indonesian infants from 6 to 12 months of age (Lind et al, 2004).

Recommendations

Further reduction of phytic acid in the mixed grain and rice infant cereals is recommended in order to insure availability of zinc for infants younger than 6 months of age. Since electrolytic iron has been shown to be approximately half well absorbed than other forms of iron, incorporation of ascorbic acid as a strong enhancer of iron is strongly recommended. Replacing the electrolytic iron and zinc sulfate by compounds with higher bioavailability and less influence on the absorption of other components of the food such as NaFeEDTA and zinc oxide is recommended.

Finally, further in vivo research is suggested to evaluate the bioavailability of iron and zinc in these samples.

References

Association of Official Analytical Chemists. (1998) Official methods of Analysis of AOAC International (16th ed.) Gaithersburg, MD: Author.

Bohn, T., Davidsson, L., Walczyk, T. & Hurrell, R. F. (2004). Phytic acid added to white-wheat bread inhibits fractional apparent magnesium absorption in humans. *American Journal of Clinical Nutrition*, 79, 418-423.

Bosscher, D., Lu, Z., Jassens, G., Van Caillie-Bertrand, M., Robberecht, H., De Rycke, H., De Wilde, R., & Deelstra, H. (2001). In vitro availability of zinc from infant foods with increasing phytic acid contents. *British Journal of Nutrition, 86*, 241-247.

Bothwell T.H. & MacPhail AP. (2004). The potential role of NaFeEDTA as an iron fortificant. *Int J Vitam Nutr Res. Nov, 74,* 421-34.

Brown, J. E., Isaacs, J. S., Krinke, U. B., Murtaugh, M. A., Stang, J., & Wooldridge, N. H. (2002). *Nutrition Through the Life Cycle*. Belmont, CA: Wadsworth.

Code of Federal Regulations. (1996). Title 21, parts 104, 170, and 184. Washington, DC. US Government Printing Office.

Conrad, M. E. & Schade, S. G. (1968). Ascorbic acid chelates in iron absorption: a role for hydrochloric acid and bile [Abstract]. *Gastroenterology,* 55, 35-45

Cook, J.D., Reddy, M.B., Burri, J., Juillerat, M.A.& Hurrell, R.F. (1997) The influence of different cereal grains on iron absorption from infant cereal foods. *American Journal of Clinical Nutrition, 65,* 964-969.

Crofton, R.W., Gvozdanovic, D., Gvozdanovic, S., Khin, C.C., Brunt, P.W., Mowat, N.A. & Aggett, P.J. (1989). Inorganic zinc and the intestinal absorption of ferrous iron [Abstract]. *Journal of Clinical Nutrition, 50*, 141-144.

Davidsson, L., Galan, P., Kastenmayer, P., Cherouvrier, F., Juillerat, M., A., Hercberg, S. & Hurrell, R.F. (1994) Iron bioavailability studied in infants: the influence of phytic acid and ascorbic acid in infant formulas based on soy isolate. *Pediatric Research, 36,* 816-822.

Davidsson, L., Almgren, A., Sandstrom, B., Hurrell, R. F. (1995) Zinc absorption in adult humans: the effect of iron fortification. *British Journal of Nutrition, 74*, 417-425.

Davidsson, L., Galan, P., Cherouvrier, F., Kastenmayer, P., Juillerat, M.A., Hercberg, S. & Hurrell, R. F. (1997). Bioavalilibility in infants of iron from infant cereals: effect of dephytinization. *American Journal of Clinical Nutrition, 65,* 916-920

Davidsson, L., Walczyk, T., Morris, A., & Hurrell R., F. (1998). Influence of ascorbic acid on iron absorption from an iron-fortified, chocolate-flavored milk drink in Jamaican children. *The American Journal of Clinical Nutrition. 67*, 873-877.

Davidsson, L., Ziegler, E., Zeder, C., Walczyk, T. & Hurrell, R. (2005). Sodium iron EDTA [NaFe(III)EDTA] as a food fortificant: erythrocyte incorporation of iron and apparent absorption of zinc, copper, calcium, and magnesium from a complementary food based on wheat and soy in healthy infants. *American Journal of Clinical Nutrition,* 81, 104-109.

Davies, N. T. & Reid, H. (1979) An evaluation of the phytate, zinc, copper, iron and manganese contents of, and Zn availability form soya-based textured-vegetable-protein meat substitutes or meat-extenders. *British Journal of Nutrition, 4I,* 579-589.

Fairweather-Tait, S. J., Wharf, S.G., & Fox, T. E. (1995). Zinc absorption in infants fed iron-fortified weaning food. *American Journal of Clinical Nutrition, 62,* 785-789.

Fomon, S. J. (1987) Bioavailability of supplemental iron in commercially prepared dry infant cereals. *Journal of Pediatrics, 110,* 660-661.

Glahn, R. P., & Wortley, G. M. (2002). Inhibition of iron uptake by phytic acid, tannic acid, and ZnCl2: Studies using an in vitro digestion/caco-2 Cell model. *Journal of Agricultural and Food Chemistry, 50,* 390-395.

Hallberg, L., Brune, M., & Rossander, L. (1986). Low bioavailability of carbonyl iron in man: studies on iron fortification of wheat flour [Abstract]. *American Journal of Clinical Nutrition, 43,* 59-67.

Haschke, F., Zeigler, E. E., Edwards, B. B., Fomon, S. J. (1986). Effect of iron fortification of infant formula on trace minerals absorption [Abstract]. *Journal of Pediatrics Gastroenterology Nutrition, 5,* 768-773.

Herman, S., Griffin, I. J., Suwarti, S., Ernawati, F., Permaesih, D., Pambudi, D. & Abrams, S.A. (2002). Cofortification of iron-fortified flour with zinc sulfate, but not zinc oxide, decreases iron absorption in Indonesian children. *American Journal of Clinical Nutrition, 76,* 813-817.

Holt, R. (1955). Studies on dried peas. I.- the determination of phytate phosphorus. *Journal of the Science of Food and Agriculture, 6,* 136-142.

Hurrell, R.F. (2002a). Enhanced iron absorption from cereal and legume grains by phytic acid degradation. *ACS Symposium Series*, 816, 117-129.

Hurrell, R F. (2002b). Fortification: Overcoming technical and practical barriers. *Journal of Nutrition, 132,* 806-812.

Hurrell, R.F. (2003). Influence of vegetable protein sources on trace element and mineral bioavailability. *Journal of Nutrition* 133, 2973s-2977s.

Hurrell, R., Bothwell. T., Cook, J.D., Dary, O., Davidsson, L., Fairweather-Tait, Hallberg, L., Lynch, S., Rosado, J., Walter, T., Whittaker, P. & SUSTAIN Task Force.(2002). The usefulness of elemental iron for cereal flour fortification: a SUSTAIN Task Force report. Sharing United States technology to aid in the improvement of nutrition. *Nutrition Reviews, 60,* 391-406.

Hurrell, R. F., Davidsson, L., Reddy, M., Kastenmayer, P. & Cook, J. D. (1998). A comparison of iron absorption in adults and infants consuming identical infant formulas. *British Journal of Nutrition, 79*, 31-36.

Hurrell, R.F., Juillerat, M.A., Reddy, M.B., Lynch, S.R., Dassenko, S.A. & Cook, J.D. (1992). Soy protein, phytate, and iron absorption in humans [Abstract]. *American Journal of Clinical Nutrition,* 56, 573-578.

Hurrell, R.F., Reddy, M.B., Juillerat, M.A. & Cook, J.D. (2003). Degradation of phytic acid in cereal porridges improves iron absorption by human subjects. *American Journal of Clinical Nutrition, 77,* 1213-1219.

Lind, T., Lonnerdal, B., Persson, L., Stenlund, H., Tennefors, C., & Hernell, O. (2003). Effects of weaning cereals with different phytate contents on hemoglobin, iron stores, and serum zinc: A randomized intervention in infants from 6 to 12 mo of age. *American Journal of Clinical Nutrition, 78*, 168-175.

Lind, T., Lonnerdal, B., Stenlund, H., Gamayanti, I.L., Ismail, D., Seswandhana, R. & Persson,L.A. (2004). A community-based randomized controlled trial of iron and zinc supplementation in Indonesian infants: effects on growth and development. *American Journal of Clinical Nutrition, 80*, 729-736.

Lind, T., Lonnerdal, B., Stenlund, H., Ismail, D., Seswandhana, R., Ekstrom, E. C. & Persson, L. A. (2003). A community-based randomized controlled trial of iron and zinc supplementation in Indonesian infants: interactions between iron and zinc. *American Journal of Clinical Nutrition, 77*, 883-890.

Lo, G. S., Settle, S. L., Steinke, F. H. & Hopkins, D. T. (1981). Effect of phytate: zinc molar ratio and isolated soybean protein on zinc bioavailability. *Journal of Nutrition, 111*, 2223-2235.

Morris, E. R., Ellis, R. (1980). Effect of dietary phytate/zinc molar ratio on growth and bone zinc response of rats fed semipurified diets. *Journal of Nutrition, 110,* 1037-1045.

Morris, E.R., Ellis, R. (1989). Usefulness of the dietary phytic acid/zinc molar ratio as an index of zinc bioavailability to rats and humans [Abstract]. *Biological Trace Element Research,19*, 107-117.

Oatway, L., Vasanthan, T., & Helm, J. H. (2001). Phytic acid. *Food Reviews International, 17*, 419-431.

Peres, J.M., Bureau, F., Neuville, D. & Arhan, P. (2001). Inhibition of zinc absorption by iron depends on their ratio [Abstract]. *Journal of Trace Elements in Medicine and Biology, 15*, 237-241.

Persson, H., Turk, M., Nyman, M., & Sandberg, A.S. (1999). Binding of Cu2+, Zn2+, and Cd2+ to inositol tri-, tetra-, penta-, and hexaphosphates [Abstract]. *Doktorsavhandlingar vid Chalmers Tekniska Hogskola. 1473*, 3194-3200.

Reddy, N.R., Pierson, M.D., Sathe, S.K. & Salunkhe, D.K. (1989). Phytates in cereals and legumes, Boca Raton, FL, CCRC Press, Inc.

Romana, D. L., Lonnerdal, B. & Brown, K.H. (2003). Absorption of zinc from wheat products fortified with iron and either zinc sulfate or zinc oxide. *American Journal of Clinical Nutrition, 78*, 279-283.

Rossander-Hulten, L., Brune, M., Sandstrom, B., Lonnerdal, B. & Hallberg L. (1991) Competitive inhibition of iron absorption by manganese and zinc in humans. *American Journal of Clinical Nutrition, 54*, 152-156.

Sandberg, A. S., Brune, M., Carlsson, N. G., Hallberg, L., Skoglund, E. & Rossander-Hulthen, L. (1999). Inositol phosphates with different numbers of phosphate groups influence iron absorption in humans. *American Journal of Clinical Nutrition, 70,* 240-246.

Sandstrom, B., Davidsson, L., Cederblad, A.& Lonnerdal, B. (1985). Oral iron, dietary ligands and zinc absorption. *Journal of Nutrition, 115*, 411-414.

Solomons, N.W. & Jacob, R.A. (1981). Studies on the bioavailability of zinc in humans: effects of heme and nonheme iron on the absorption of zinc [Abstract]. *American Journal of Clinical Nutrition, 34,* 475–482.

Walter, T., Pizarro, F., Abrams, S. A., Boy, E. (2004). Bioavailability of elemental iron powder in white wheat bread [Abstract]. *European Journal of Clinical Nutrition, 58,* 555-558.

Wise, A. (1995). Phytate and zinc bioavailability [Review] [Abstract]. *International Journal of Food Science and Nutrition, 46,* 53-63.

Whittaker, P. (1998). Iron and zinc interactions in humans [Review]. *American Journal of Clinical Nutrition*, 68, 442S-446S.

World Health Organization, Complementary feeding. Retrieved March 30, 2004, from http://www.who.int/child-adolescent-health/NUTRITION/complementary.htm

Zhao, X.F., Hao, L.Y., Yin, S.A., Kastenmayor, P. & Barclay, D. (2003). A study on absorption and utilization of calcium, iron and zinc in mineral-fortified and dephytinized soy milk powder consumed by boys aged 12 to 14 years [Abstract]. *Zhonghua Yu Fang Yi Xue Za Zhi., 37,* 5-8.